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INTERACTION OF LEUKOTRIENE C_4 AND CHINESE HAMSTER LUNG FIBROBLASTS (V79A03 CELLS). 2. SUBCELLULAR DISTRIBUTION OF BINDING AND UNLIKELY ROLE OF GLUTATHIONE-S-TRANSFERASE

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Abstract

It was reported previously that radiation-induced cytotoxicity in V79A03 (V79) cells was attenuated by pretreatment of cells with leukotriene C_4 (LTC_4), leading us to determine that V79 cells possessed specific binding sites, with characteristics of receptors, for LTC_4 (see the preceding, companion communication). Additional studies were conducted to determine the subcellular distribution and the chemical nature of the LTC_4 binding site in V79 cells. Trypsin treatment of cells before LTC_4 binding assays resulted in a 74% reduction in high-affinity binding. In tests to examine the subcellular location of LTC_4 binding, plasma membrane and nuclear fractions were obtained from V79 cells. In contrast to Scatchard analyses of LTC_4 binding to intact cells which were curvilinear, Scatchard analyses of nuclear and plasma membrane fractions were linear, indicative of the presence in these cellular substituents of low and high-affinity binding, respectively. To examine the nature of the high-affinity LTC_4 binding sites, intact V79 cells were photolyzed with [^{3}H]-LTC_4 rendered photoactive by preincubation with N-hydroxysuccinimidyl-4-azidobenzoate. The cell-bound radioactivity migrated during sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with an apparent molecular weight of approximately 40 kdal. Five different commercial preparations of glutathione-S-transferase (GST), which has been implicated as a source of LTC_4 "specific binding" in other cells, migrated in the same SDS-PAGE system with an apparent molecular weight of 20-24 kdal. Furthermore, preincubations of V79 cells with three antisera generated against GST had minimal effects upon subsequent LTC_4 binding to intact cells. These data, taken together with the data from the preceding companion communication, suggest that the radioprotective effect of LTC_4 upon V79 cells may be attributable to a receptor-mediated phenomenon which appears distinct from leukotriene binding to GST.

Introduction

The cytotoxic effects of γ-irradiation from a ^{60}Co source were attenuated in V79A03 (V79) cells that were pretreated with leukotriene C_4 (LTC_4) (1), which induced us to demonstrate that V79 cells contained binding sites for [^{3}H]-LTC_4 (2). Interaction of LTC_4 with V79 cells was reminiscent of a ligand-receptor interaction, because LTC_4 bound to V79 cells in a manner that was specific, reversible, and with both a high- and low-affinity component. However, specific binding of LTC_4, without a demonstrable biological role for the leukotriene, has been reported for several cells,

cell lines, and tissues (reviewed in 3), leading to questions of the biological significance of widespread LTC_4 binding phenomena. The widely distributed detoxicating enzyme glutathione-S-transferase (GST) has been implicated in LTC_4-binding phenomena, following the demonstration by Sun et al. (4) that LTC_4 bound with high-affinity to GST in rat liver.

To further characterize the interaction of LTC_4 with V79 cells, we examined the leukotriene-binding characteristics of plasma membrane and nuclei fractions from V79 cells. To examine the possibility that binding of LTC_4 to V79 cells could be attributed to GST, we examined the mobilities on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the photolytic product of V79 cells and [^{3}H]-LTC_4 derivatized with the bifunctional reagent N-hydroxysuccinimidyl-4-azidobenzoate (HSAB), and monitored the effect of antibodies against GST upon LTC_4 binding to V79 cells.

Methods

Materials. [14,15-^{3}H] Leukotriene C_4 ([^{3}H]-LTC_4, 38.4 Ci/mmol) and ^{125}I-Na were purchased from Dupont-NEN (Boston, MA). Nonradioactive LTC_4 was obtained from by Cayman Chemical (Ann Arbor, MI). HSAB and bicinchoninic acid protein assay kit were purchased from Pierce Chemical (Rockford IL). Supplies for SDS-PAGE were purchased from Bio-Rad (Richmond, CA). GST, protein molecular weight standards, L-serine and 5'-nucleotidase kit were obtained from Sigma (St. Louis, MO). Antisera against GST were a gift from Dr. W. Jakoby (National Institutes of Health, Bethesda, MD).

LTC_4 Binding Assay. Binding of [^{3}H]-LTC_4 to preparations of V79 cells was assessed using the methodology described in the preceding companion communication (2), with modifications as described. Aliquots of plasma membrane, cells or isolated nuclei were incubated for 30 min on ice in Hank's buffered salt solution (HBSS) containing 25 mM HEPES, pH 7.35, and 20-70 fmol of [^{3}H]-LTC_4. Nonspecific binding was determined in triplicate by the addition of 16 μM unlabeled LTC_4 to a parallel set of assay tubes. Displacement curves were generated using incubations in graded levels of LTC_4 from 0 to 16 μM. Binding assays were terminated by dilution with 3 ml of ice-cold HBSS and the bound radioactivity was recovered by filtration through Whatman GF/B filters on a Yeda filtration manifold. Bound radioactivity was assessed on a Tracor Analytic Mark III scintillation counter. All binding assays were conducted in duplicate or triplicate, and all experiments were repeated at least once. To assess LTC_4 binding by preparations of plasma membranes or nuclei, preliminary experiments were conducted to demonstrate that radioactivity retention by Whatman GF/B filters was equivalent to retention following filtration on Millipore HAWP, EHWP or GSWP disks, or following centrifugation at approximately 14,000 x g in a microfuge (Eppendorf; Brinkman Instruments; Westbury, NY).

Enzymatic Modification of Glycoproteins. Lots of 40-60 x 10^6 V79 cells were harvested by exposure to PBS-EDTA, divided into equal fractions, and pelleted by centrifugation at 1000 x g. The pelleted cells were resuspended in 10 ml PBS-EDTA containing (a) no enzymes (control), (b) 10 mg hyaluronidase, (c) 1 mg neuraminidase, or (d) 2.5 μg trypsin. The cells were incubated in these solutions for 5 min at 22°C, repelleted, resuspended in IMDM containing 5% fetal bovine serum (FBS) and cooled to

4°C. This medium was replaced with the assay buffer; then Scatchard analyses were conducted using control cells and cells from each enzyme treatment.

Preparation of Nuclei and Plasma Membranes. Fractions highly enriched in nuclei and plasma membranes were prepared by the aqueous two-phase polymer system of Lesko (5). Briefly, harvested cells were resuspended in swelling buffer which consisted of 0.5 mM $CaCl_2$ and 1.0 mM $NaHCO_3$, at pH 7.5 to which 1 mg/l of deoxyribonuclease was added. The cells were allowed to equilibrate in swelling buffer for approximately 30 min during which time the nuclei became swollen. The plasma membranes were lysed using a Dounce homogenizer. The homogenate was centrifuged at 10,000 x g to pellet the plasma membrane and nuclear fractions. The pellet was resuspended and the nuclei separated from the plasma membrane using the aqueous two-phase polymer system. Plasma membranes were retained at the polymer interface while the nuclei were isolated in the pellet. Both fractions were washed twice in HBSS to remove the polymer mixture, prior to use of the fractions in binding assays. Binding assays were conducted using approximately 1-5 x 10^6 nuclei or 10-50 μg of membrane protein per assay tube.

Cell Surface Iodination. To monitor the possible contamination of isolated nuclei with cell surface components, nuclei were prepared from cells in which a portion was cell-surface radioiodinated. Intact viable V79 cells harvested by incubation with 0.002% EDTA were labeled with ^{125}I using lactoperoxidase catalysis modified from the procedure of Miyachi et al. (6). Briefly, ten million cells were suspended in HBSS-HEPES (pH 7.4, 200 μl volume), to which 1 mCi Na-^{125}I, 8 μg lactoperoxidase and 800 ng H_2O_2 were added. Iodination was allowed to proceed for 5 min at 22°C, then cells were washed twice to remove free iodine. Cell-bound radioactivity was assessed by solid scintillation counting. The iodinated cells were added to 10^{10} unlabeled V79 cells and the nuclei isolated as described above.

5'-Nucleotidase Assay. 5'-Nucleotidase activity was assayed by the method of Arkesteijn (7). Whole cells, nuclei, and purified plasma membrane were resuspended in distilled water and homogenized using a Brinkman Polytron. The protein content of each fraction was determined with a bicinchoninic acid protein assay kit, and each solution was diluted with water to a concentration of 1 mg/ml. 5'-Nucleotidase activity was assayed in quadruplicate using approximately 70 μg of protein per assay tube.

Preparation of (Azidobenzoyl) [^{3}H]-LTC_4. The ethanol in 180 μl of stock [^{3}H]-LTC_4 (1.8 μCi, 46.8 pmol) was evaporated under a stream of nitrogen gas and 1 M K_2HPO_4, pH 8.0 was added to the remaining aqueous solution to produce a final concentration of approximately 0.52 μM LTC_4. Two hundred nmol HSAB in 10 μl of dimethyl sulfoxide was added to the [^{3}H]-LTC_4 solution, mixed and incubated in the dark at 22°C for 60 min.

Crosslinking of (Azidobenzoyl) [^{3}H]-LTC_4 to V79 Cells. Freshly harvested V79 cells ($5x10^6$) were washed twice and incubated in 10 mM serine-borate/HBSS, pH 7.4 with 26 nM (azidobenzoyl) [^{3}H]-LTC_4 in a final volume of 900 μl. After incubation at room temperature for 90 min in a dark room, the mixture was pelleted by centrifugation at 1500 x g for 2 min, followed by aspiration of the supernatant. The cell pellet was irradiated

using a 254 nm UV lamp (Spectroline, Model ENP-26, Spectronics Corporation, NY) at 1.5-2.0 cm distance at 22°C for 6 min. The cells were suspended in 1 ml of 10 mM serine-borate/HBSS, pH 7.4, and centrifuged at 1500 x g for 5 min. The resulting pellet was collected for electrophoresis. The crosslinking protocol was also replicated using one of two modifications to assess nonspecific binding; addition of 14 μM unlabeled LTC_4 or omission of the UV irradiation step.

SDS-PAGE. The cell pellet was solubilized in 200 μl of SDS-PAGE sample buffer (2% SDS, 10% glycerol, 0.001% bromphenol blue, 50 mM Tris-HCl, 8 M urea with reducing agents 50 mM dithiothreitol and 2% β-mercaptoethanol), by boiling for 5 min. The soluble material was obtained by centrifugation in a Beckman Type 75TI rotor at 100,000 x g for 45 min at 20°C. 130-150 μl sample was loaded onto the gel. A 5-20% linear gradient acrylamide gel was prepared using the procedure of Laemmli (8). Gels contained individual lanes of non-irradiated sample, samples incubated in the absence or presence of 14 μM LTC_4, and molecular weight markers. After electrophoresis, the gel was cut into lanes, and selected lanes were cut into 0.5 cm sections. Each section was immersed in 10 ml of Hydrofluor, then homogenized by polytron (Beckman Instruments, NY; setting 10) for 10 sec. Radioactivity in homogenized sections was quantified by β-scintillation detection. Proteins were visualized in selected lanes using Comassie blue stain. This experiment was replicated three times with equivalent results. The same electrophoretic methodology was utilized to assess the electrophoretic mobility of commercially available preparations of GST obtained from human placentas and livers of bovine, equine, rabbit, and rat.

Incubations with Anti-GST Antisera. LTC_4 binding assays were conducted in the presence of antisera against isoforms of GST (anti-B, anti-C, and anti-E; kindly provided by Dr. W. Jakoby). Binding assays were conducted as described earlier, except that cells in assay tubes were preincubated for 10 min at 22°C in assay buffer containing 10% (v/v) antiserum before addition of [^{3}H]-LTC_4. Each antiserum was evaluated at full strength, at 1:10 and 1:100 dilutions, and compared with simultaneous, control incubations conducted in the absence of antiserum.

GST Assay. GST activity was determined using homogenates of V79 cells prepared by sonication, and using plasma membranes that were purified according to the procedure described by Lesko *et al*. (5). GST activity was measured using 1-chlor-2,4-dinitrobenzene as a substrate (4). Assays were performed in quadruplicate.

Results

Enzyme Treatments. V79 cells were incubated with enzymes capable of modifying glycoproteins. No treatment significantly influenced binding to the low-affinity binding site. However, treatment with trypsin reduced LTC_4 binding to the high-affinity binding site to 26% that of controls (Table 1). Treatment with neuraminidase slightly enhanced LTC_4 binding, while treatment with hyaluronidase caused increased, but variable LTC_4 binding. Examination of the cells under a light microscope revealed that the cells treated with the hyaluronidase were swollen and partially lysed. Extended periods of trypsinization also resulted in cell lysis and a partial restoration of binding (data not shown). It is possible that the

Table 1. Effect of Enzymes on [^{3}H]-LTC$_4$ Binding.[a]

Enzyme Treatment	% Control Binding[b]
None (EDTA)	100
Hyaluronidase (N=3)[c]	181 ± 54
Neuraminidase (N=3)	134 ± 8[d]
Trypsin (N=5)	26 ± 10[d]

[a] V79 cells were incubated with enzymes as described in Methods. After enzyme treatment, binding of [^{3}H]-LTC$_4$ was assessed by Scatchard analyses, and high affinity sites were quantified using LIGAND

[b] ($\bar{x}$ ± SEM)

[c] N represents the number of separate experiments

[d] P<0.01 by t-test

increases observed after treatment with several enzymes were due to the release of intracellular binding sites rather than to enzymatic modification of the cell surface binding sites. No effect of enzyme treatment upon binding affinities was apparent.

Preparations of V79 Nuclei and Plasma Membranes. Preparations of nuclei were assessed by determination of 5'-nucleotidase and by monitoring retention of cell-surface radioiodine. Preliminary experiments with iodination using nonradioactive iodine indicated a negligible effect of the iodination procedure upon cellular integrity and viability, as assessed by exclusion of trypan blue. Using the commercial kit for 5'-nucleotidase, we could not detect this enzyme in nuclear preparations. By monitoring radioactivity in nuclei isolated from radioiodinated cells, up to 12% of the radioiodine present on intact cells was detected in the nuclear preparation. The plasma membrane fraction used in these studies was that remaining at the polymer interface during final pelleting of nuclei in the two-phase polymer system. Plasma membranes appeared free of nuclei by microscopic examination, but other assessments of plasma membrane purity were not attempted.

LTC$_4$ Binding to V79 Subcellular Fractions. Scatchard analyses were conducted of LTC$_4$ binding to plasma membrane and nuclear fractions from V79 cells. A representative plot of LTC$_4$ binding to plasma membranes is depicted in Fig. 1. Using LIGAND, the data were best fit by a straight line, suggestive of a single class of binding sites having a Kd ≈ 43 nM. A depiction of LTC$_4$ binding to two concentrations of nuclei is presented in Fig. 2. The binding of LTC$_4$ to nuclei were best fit by a straight line indicating a Kd ≈ 240 nM. Scatchard analyses of LTC$_4$ binding to both plasma membranes and nuclei were repeated twice with equivalent results.

Association and dissociation kinetics of LTC$_4$ binding to isolated nuclei were compared to that obtained using intact cells (Fig. 3). The association of LTC$_4$ to 10^6 nuclei proceeded rapidly but attained a lower magnitude than LTC$_4$ binding to an equivalent number of intact cells, while dissociation of LTC$_4$ from nuclei followed a similar time course as dissociation from intact cells.

Crosslinking of LTC$_4$ to V79 Cells. The reaction product of [^{3}H]-LTC$_4$ and HSAB retained the capacity to bind to V79 cells (Fig. 4). The electrophoretic mobility of radioactive photoreaction products is depicted in Fig. 5, which was representative of three replicated experiments. A major radioactive peak was demonstrable which corresponded to a molecular mass of 40 kDa. When cells were incubated with [^{3}H]-LTC$_4$ in the presence

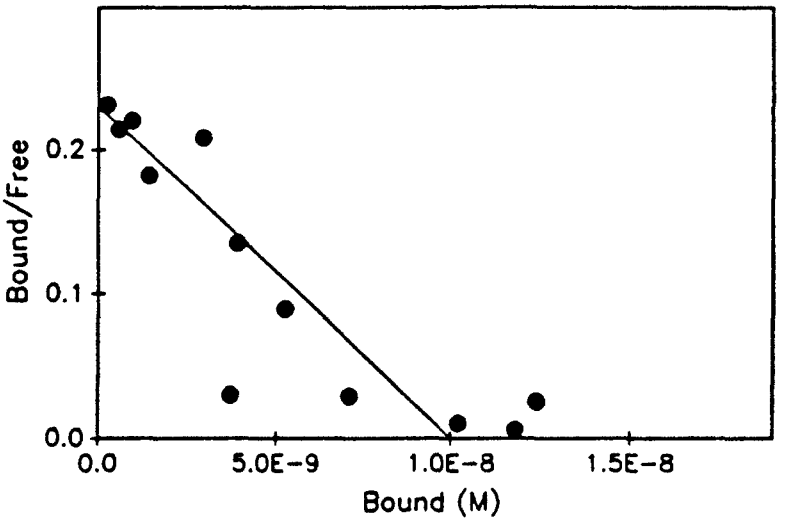

Fig. 1. Scatchard Plot of LTC_4 Binding to V79 Cell Plasma Membranes. Membranes were prepared as described in Methods. Scatchard analyses of LTC_4 binding to approximately 1 mg membrane protein/tube were conducted as described in Methods.

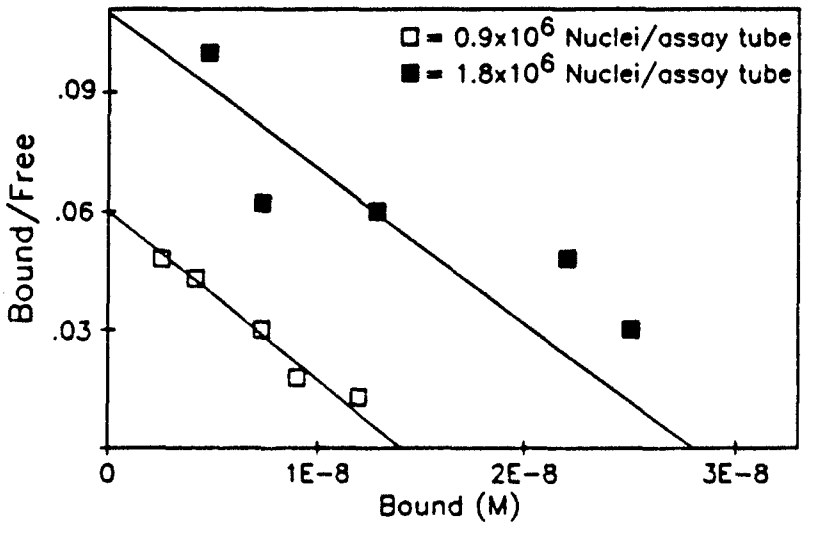

Fig. 2. Scatchard Plots of Leukotriene C_4 (LTC_4) Binding to V79 Nuclei. Nuclei preparation and Scatchard analyses were performed as described in Methods. Binding assays were conducted at two concentrations of nuclei.

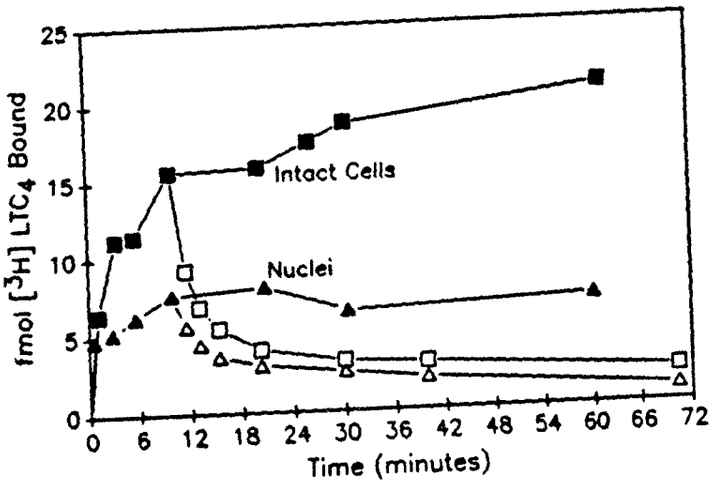

Fig. 3. Association and Dissociation of LTC_4 to V79 Intact Cells and Nuclei. Tubes containing 10^6 cells (•) or 10^6 nuclei (▲) per tube were incubated at 4°C with 1.3 nM [^{3}H]-LTC_4 in 50 µl assay buffer for the indicated times. Dissociation was monitored (open symbols) in tubes to which LTC_4 (16 µM final concentration) was added at 10 min. Incubations were terminated by filtration.

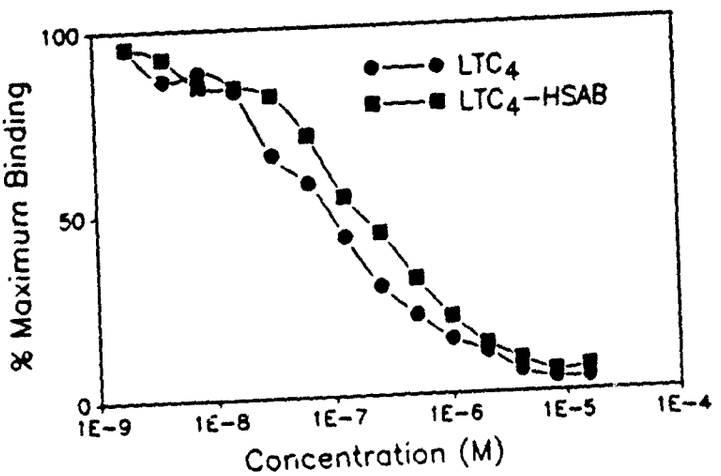

Fig. 4. Displacement of [^{3}H]-LTC_4 Binding to V79 Cells by LTC_4 and LTC_4 Derivatized with HSAB. Binding assays were conducted as described in Methods. In one set of tubes (•), indicated doses of LTC_4 were added. In the other set (▪), indicated doses of LTC_4 obtained after derivatization were added.

of 14 μM nonradioactive LTC_4, radioactivity in the gel was greatly reduced (Fig. 5). When samples were not irradiated, radiation in gel fractions was also reduced to levels similar to nonspecific binding (data not shown).

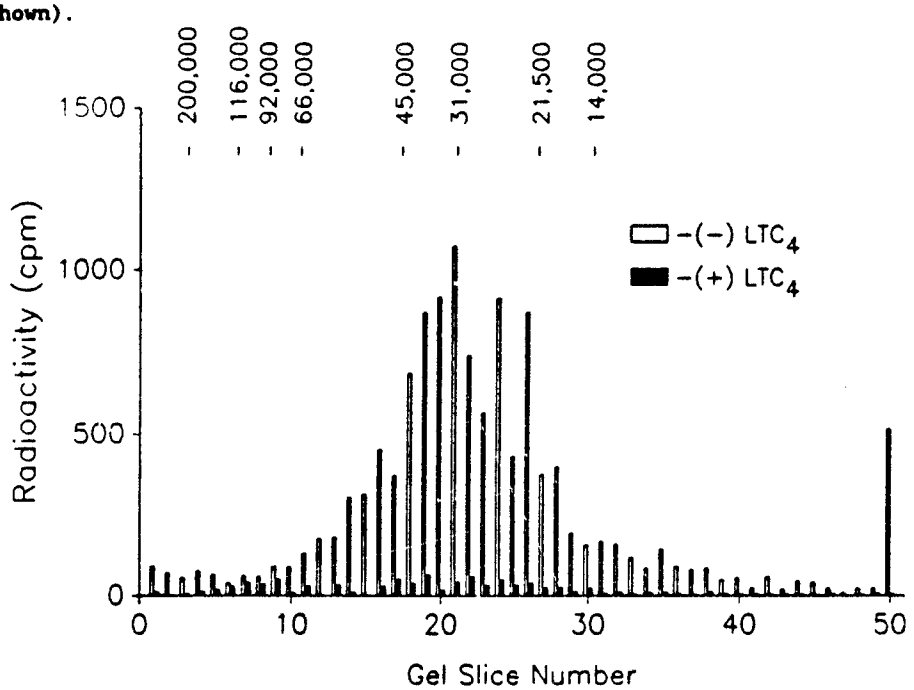

Fig. 5. Radioactivity in SDS-PAGE Slices Following Crosslinking of [^{3}H]-LTC_4 to V79 Cells. [^{3}H]-LTC_4 was crosslinked to cells as described in Methods, in the absence (open bars) and presence (solid bars) of excess unlabeled LTC_4. Gel lanes were sectioned, homogenized, and subjected to β-scintillation counting. The migration positions of the molecular weight markers are indicated.

Using electrophoretic conditions identical to those used to identify photoreaction products, the mobility of five different commercial preparations of GST corresponded to approximately 22-25 kDa (Fig. 6). The data of Figs. 5 and 6, taken together, are suggestive that the major binding product resulting from LTC_4 crosslinking is not GST.

GST Activity in V79 Cell Fractions. As another avenue to assess the possibility that GST could account for LTC_4 binding to V79 cells, GST activity was examined in homogenates prepared by sonication and in plasma membranes. Summarizing data from three different determinations (each determination in triplicate), enzyme activity in sonicated V79 cell preparations was 2.91 ± 0.61 mIU/mg protein, and enzyme activity in the plasma membrane fraction was undetectable (lower limit of detection approximately 0.04 mIU/mg protein). Homogenized cells bound 26 ± 0.9 fmol LTC_4/mg protein and plasma membranes bound 31.4 ± 2.07 fmol LTC_4/mg protein. In comparison, a commercially obtained standard of GST from bovine liver bound approximately 5 fmol LTC_4 per unit of enzyme activity.

Accordingly, GST appeared to be a possible source of a small amount of LTC_4 binding to V79 cells, but appeared unlikely to account for the magnitude of LTC_4 binding observed.

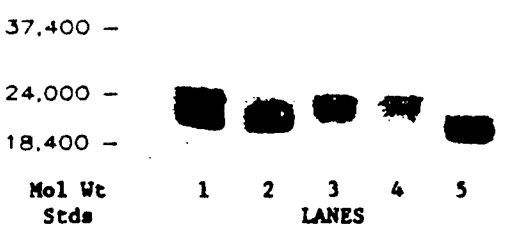

Fig. 6. SDS-PAGE of Five Different Sources of GST. Preparations of GST were solubilized and eluted using the same conditions described for Fig. 5. Sources of GST were Lane 1=rat liver, Lane 2=equine liver, Lane 3=rabbit liver, Lane 4=bovine liver, Lane 5=human placenta.

Further evidence indicating that GST was not the major binding site in intact V79 cells was obtained using binding assays in the presence of antisera against GST. When LTC_4 binding assays were conducted in the presence of 0.1, 1, or 10% dilutions of anti-B, anti-D or anti-E antisera, no influence upon [^{3}H]-LTC_4 binding was detected (data not shown).

Discussion

We conducted experiments to characterize LTC_4 binding to V79 cells. In the report which precedes this communication (2), information was provided relative to the binding characteristics of LTC_4 and viable V79 cells. In this communication, we evaluated the subcellular distribution of LTC_4 binding to V79 cells and examined the possibility that GST was the principal source of LTC_4 binding to V79 cells.

Scatchard analyses of LTC_4 binding to intact V79 cells resulted in concave plots (2), suggesting either that the cells contained multiple classes of binding sites, or that the binding sites displayed negative binding cooperativity. The former possibility appears more likely because curvilinear Scatchard plots resulted from assays of LTC_4 binding to intact cells but linear plots were obtained in assays using preparations of plasma membrane or nuclei, and Hill plots of LTC_4 binding to V79 cells or subcellular components were consistently equal to or less than unity (data not shown). Consequently, it appears probable that V79 cells contain both high-affinity and low-affinity binding sites.

The LTC_4 binding sites appeared proteinaceous in nature because preincubations of V79 cells with trypsin greatly attenuated subsequent LTC_4 binding (Table 1). The reaction product of a photoactive derivative of LTC_4 and intact V79 cells exhibited mobility on SDS-PAGE of approximately 40 kDa (Fig. 5). The high-affinity LTC_4 binding site appeared to be localized largely to the V79 cell surface, because intact, viable cells contained abundant binding and no increase in high-affinity binding was detected when cells were homogenized before LTC_4 binding assays (data not shown). Preparations enriched in plasma membranes also possessed high-affinity binding without a demonstrable low-affinity component (Fig. 1). Conversely, only low-affinity LTC_4 binding was detected using isolated V79 cell nuclei (Fig. 2). Preparations of nuclei had no detectable 5'-

nucleotidase activity, but contained up to approximately 12% of the radioiodine incorporated onto intact cells, suggesting that nuclei might have been contaminated with a small but demonstrable amount of the cell surface.

Interaction of LTC_4 with V79 cells was previously correlated with a quantifiable biological response, namely protection against γ-irradiation (1). Thus, V79 cells that were pretreated with LTC_4 proved resistant to subsequent γ-irradiation. Our studies of LTC_4 binding to V79 cell preparations suggest that the radioprotective effect of LTC_4 can be attributed to a receptor-mediated event. LTC_4 is a thiol-ether and thiols are known to act as radioprotectants through several mechanisms including free-radical scavenging. However, the concentrations of LTC_4 that induce radioprotection are much lower than necessary for the most effective thiol radioprotections (9). As demonstrated in the companion communication (2), LTC_4 binding to V79 cells is of high-affinity, specific, saturable, and reversible. We report studies in this communication demonstrating that LTC_4 binding appears to be attributable to a $\approx$40,000 molecular weight protein which is localized at least in part on the plasma membrane of V79 cells. Finally, the concentrations of LTC_4 that conferred radioprotection (1) correlated with the high-affinity binding of LTC_4 to V79 cells.

The mechanism by which LTC_4 influences the function of its target tissues presumably proceeds by a receptor-mediated process (10): accordingly, specific binding sites for LTC_4 have been reported on several putative target tissues, including guinea pig lung (11), brain and uterus (12), myocardial membranes (13), human lung (14,15), rat lung (16), rat renal glomeruli (17), and on several smooth muscle cell lines (18,19). However, many of these studies did not describe the observed specific binding as a receptor because correlations between LTC_4 binding and target tissue response were not established.

V79 cells are derived from Chinese hamster lung fibroblasts. A LTC_4 receptor was also described in lung fibroblasts from rats (20). The rat fibroblast receptor had many similarities to the binding site which we report in V79 cells, although some differences in the two systems were apparent (such as cyclic nucleotide dependence, and activity of the leukotriene antagonist FPL 55712) which might be attributable to species differences, degree of cell passage, or methodological differences. However, it is most interesting that LTC_4 binding to the rat receptor was correlated with stimulation of collagen synthesis, leading Phan *et al.* (20) to speculate that LTC_4 might serve a cytoprotective or wound healing function. Thus, it is possible that the conferrence of radioprotection to V79 cells by LTC_4 treatment proceeds via a receptor-mediated effect upon protein synthesis. However, we did not address this possible pathway.

We examined the possibility that GST was the source of LTC_4 binding in V79 cells. The glutathione transferases are a multigene family of isoenzymes that catalyze the conjugation of glutathione to electrophilic compounds as the first step in a detoxification pathway (21). Sun *et al.* (4) provided convincing evidence that GST was a major source of LTC_4 binding to rat liver cells, but later urged caution in ascribing physiological significance to LTC_4 binding by tissues and cells for which a physiological role of LTC_4 has not been described (3).

The family of enzymes which are collectively designated GST has been detected in a variety of tissues including lung, kidney, and testis, but is most abundant in liver, where it may constitute 10% of the total soluble protein (22). The glutathione transferases serve in detoxication and have the capacity to bind an enormous number of compounds that have a hydrophobic character (22). It is interesting to speculate that LTC_4 may bind V79 cells (and possibly other cells and tissues) by binding to GST. LTC_4 binding to GST might also account for the inhibition by PGA_2 of LTC_4 binding to V79 cells (4). because GST has been reported to catalyze the conjugation of PGA_1 and glutathione (23).

One interpretation of the data for the two LTC_4 binding sites on V79 cells is that one site represents GST and the other represents γ-glutamyl transpeptidase. γ-Glutamyl transpeptidase, which catalyses the conversion of LTC_4 to leukotriene D_4 (LTD_4), seems an unlikely candidate for a major site of LTC_4 binding to V79 cells: (a) Serine-borate was routinely added to incubates to inhibit the activity of this enzyme. (b) Conversion of LTC_4 to LTD_4 by V79 cells incubated under standard assay conditions could not be detected (Fig. 5 in reference 2). (c) The reported molecular weights (46,000 and 22,000 for two subunits) and binding affinity (Kd = 5-10 μM) of γ-glutamyl transpeptidase (24) are at variance with the values obtained for the interaction of LTC_4 with V79 cells (2, this report). We also think it unlikely that GST is a major source of LTC_4 binding in V79 cells: (a) Intact cells were used for LTC_4 binding studies. GST is predominantly intracellular, and was detectable in homogenates of V79 cells (see Results) but not in plasma membrane preparations, although plasma membranes serve as an effective source of LTC_4 binding. Passive cellular internalization of LTC_4 (which is water soluble) to provide access to intracellular GST would be unexpected, and an active process of LTC_4 internalization seems unlikely during our incubations at 4°C. (b) In the event that LTC_4 traversed the V79A03 cell membrane and bound to intracellular GST, the magnitude of detectable GST activity in homogenized cells appeared insufficient to account for all LTC_4 binding (see Results). (c) Preincubations of intact V79 cells with antisera against GST isozymes B, C and E had no effect upon binding of LTC_4 (data not shown). (d) Photolysis of a photoreactive derivative of LTC_4 and V79 cells resulted in a product that migrated on SDS-PAGE with an apparent molecular weight of 40 kDa, while several commercial preparations of GST migrated in the same gel system with an apparent molecular weight of 20-24 kDa. (e) Given the capacity of GST to bind a multitude of molecules having some degree of hydrophobicity, no mechanism has been advanced by which binding of LTC_4 by GST would confer radioprotection. Ligands bound by GST serve to inhibit enzymic activity (25), thus it is difficult to propose a mechanism by which occupancy (and inactivation) of the detoxicating enzyme GST by LTC_4 would promote radioprotection.

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